

Contents

List of protocols xiii

Abbreviations xvii

Introduction: zebrafish as a system to study development and organogenesis 1

Christiane Nüsslein-Volhard, Darren T. Gilmour, and Ralf Dahm

1 History 1

2 Phylogeny 2

3 The zebrafish genome 2

4 Advantages of the zebrafish as a model system 4

5 Purpose of this book 4

Acknowledgements 5

References 5

1 Keeping and raising zebrafish 7

Michael Brand, Michael Granato, and Christiane Nüsslein-Volhard

1 Introduction 7

2 Aquaria systems and water conditions 7

Aquaria systems 7

Aquaria 10

3 Water 12

Fresh fish water 12

Filtering 14

4 Room conditions and maintenance 15

Room conditions 15

Temperature 16

Illumination 16

Cleaning 16

Safety 18

5 Fish care 18

Keeping adult fish 18

Raising fish 19

6 Maintaining stocks 28

Maintaining wild-type stocks 28

Maintaining mutant stocks 28

Identifying and maintaining adult heterozygous mutant carriers 29

Maintaining mutant stocks as frozen sperm 30

CONTENTS

- 7 Fish health 34
 - Fish diseases 34
 - Fish handling in systems with latent TB 35
 - Quarantine 36
 - References 37

2 Looking at embryos 39

Stefan Schulte-Merker

- 1 Introduction 39
- 2 *In situ* hybridization 39
 - Single probe 40
 - Detecting two differently labelled probes *in situ* 43
- 3 Antibody staining 45
 - Detecting antigens in early embryos using biotinylated secondary antibodies 45
 - Antibody staining of zebrafish larvae 47
 - Double *in situ* hybridization combined with antibody staining (triple stain) 48
 - Pre-absorption of antibodies 50
- 4 Mounting 51
 - Viewing chambers for observing embryos 51
 - Methyl cellulose mounting 51
 - Araldite mounting 52
 - Mounting in benzyl benzoate/benzyl alcohol 53
- 5 Preparing sections 53
 - Plastic sections using Technovit® 54
 - Paraffin sections combined with antibody staining 54
 - Embedding and sectioning using JB-4 resin 55
 - Toluidine Blue staining of semi-thin sections 56
- 6 PTU treatment to prevent melanization of embryos 57
- 7 Endothelial cell staining 57
- Acknowledgements 58
- References 58

3 The morphology of larval and adult zebrafish 59

Thomas F. Schilling

- 1 Introduction 59
- 2 Body proportions and surface features 59
 - Adult 59
 - Larvae and juveniles 61
- 3 Skeleton and musculature 65
 - Adult skeleton 65
 - Larval and juvenile skeletal development 69
 - Adult musculature 73
 - Larval and juvenile muscle development 74
- 4 Nervous system 76
 - Adult central nervous system 76
 - Larval and juvenile neural development 79
 - Adult peripheral nervous system and sense organs 83
 - Larval and juvenile development of the PNS and sense organs 86

5 Cardiovascular, digestive, and reproductive organs	87
Adult	87
Larval and juvenile organogenesis	91
Acknowledgements	92
References	92
4 Cell labelling and transplantation techniques	95
<i>Donald A. Kane and Yasuyuki Kishimoto</i>	
1 Introduction	95
2 Cell labelling techniques	96
Labelling whole embryos with lineage tracer dyes	97
Labelling individual blastomeres after 256-cell stage	101
Labelling groups of cells by photo-activation	102
Labelling groups of cells by lipophilic membrane dyes	103
3 Transplantation techniques	103
Precision transplantation of small groups of cells	104
Large-scale transplantations of early blastomeres	108
Transplantation of pieces of embryos	109
4 Procedures for observing labelled cells	110
Procedures for observing live material	111
Procedures for preparing fixed material	114
Procedures for analysing data	117
References	119
5 Manipulating gene expression in the zebrafish	121
<i>Darren T. Gilmour, Jason R. Jessen, and Shuo Lin</i>	
1 Introduction	121
2 Microinjection of zebrafish embryos	121
Equipment required	122
The microinjection procedure	125
3 Transient expression approaches	128
DNA constructs and mRNA transcripts give different expression profiles upon injection	129
RNA injection	129
DNA microinjection and its applications	131
Promoter analysis	132
Co-injection	133
Rescue of mutants using transgenesis	134
Artificial chromosome transgenesis in zebrafish	134
4 Generation of stable germ line transgenic lines	137
Identifying transgenic founder fish by PCR	138
Identification of transgenic founder fish by reporter gene expression	139
Generation of homozygous transgenic fish	140
5 Morpholino 'knockdown' of gene activity	141
References	143

6 Mutagenesis 145

Francisco Pelegri

- 1 Introduction 145
- 2 Mutagenic agents 145
 - Chemical mutagens: N-ethyl-N-nitrosourea 145
 - Radiation sources 149
 - Insertional mutagenesis 150
- 3 Selection of background genetic lines 152
 - General criteria for selecting genetic backgrounds 152
 - Selection of lines amenable for *in vitro* fertilization and parthenogenesis 1520
 - Selection of lines free of lethal or sterile mutations 153
- 4 Genetic screening strategies 153
 - Inbreeding of families carrying non-mosaic germ lines 153
 - Screens involving ploidy manipulation 156
 - Screens for recessive mutations affecting adult traits and maternal-effect mutations 167
 - Screens for dominant mutations 167
 - Allele screens 167
- 5 Screen tests 172
 - Morphological screens 172
 - Screens using molecular markers and tissue stains 172
 - Locomotion and behavioural screens 172
- 6 Conclusions 172
 - Acknowledgements 173
 - References 173

7 Mapping and cloning 175

Robert Geisler

- 1 Maps of the zebrafish genome 175
 - 2 Mapping mutations using SSLP markers 177
 - Mapping approaches 177
 - Selection of zebrafish lines and SSLPs 178
 - Mapping strategy 180
 - Set-up for high-throughput mapping 183
 - Evaluating pooled PCR gels 185
 - Evaluating single-embryo PCR gels 186
 - Calculation of a map position 189
 - Fine mapping 192
 - 3 Radiation hybrid mapping of candidate genes 192
 - 4 Matching mutations with candidate genes 195
 - Searching maps and databases 195
 - Evidence for the identity of a mutant locus 196
 - Demonstrating a direct linkage by SNP genotyping 196
 - 5 Positional cloning of mutations 199
 - Genomic libraries 199
 - Screening for genomic clones 201
 - Analysing a genomic clone 207
- Acknowledgements 210
- References 210

Appendix 1 List of suppliers 213

Appendix 2 Atlas of embryonic stages of development in the zebrafish 219

Ralf Dahm

Appendix 3 Table of zebrafish mutations 237

Hans Georg Frohnhofer

Index 293